REMARKS

Status

Claims 1-3, 8, 10, 12, 13, 21, 30, 31, 68 and 71 were at issue in this Office Action. The present response does not add or delete any claims.

The Office Action

In the Office Action mailed July 15, 2008, all claims were rejected under 35 U.S.C. §112, second paragraph, as being indefinite.

Claims 1, 8, 10, 13, 21, 30, 31, 68 and 71 were rejected under 35 U.S.C. §102 as being anticipated by U.S. Patent 4,649,114 of Miltenburger.

Claims 1-3, 8, 10, 21, 30, 31, 68 and 71 were rejected under 35 U.S.C. §102 as being anticipated by the publication of Wodnicka.

Claims 1-3, 8, 10, 21, 30, 31, 68 and 71 were rejected under 35 U.S.C. §103 as being unpatentable over the Miltenburger '114 patent. Claims 1-3, 8, 10, 12, 13, 21, 30, 31, 68 and 71 were rejected under 35 U.S.C. §103 as being unpatentable over the '114 patent taken in view of the publication of Schwarz et al.

Applicant thanks the Examiner for the Office Action, for the thorough explanation of the basis of the rejections, and for the withdrawal of particular rejections previously made.

The Rejections under 35 U.S.C. §112

Applicant respectfully submits that in view of the amendment to the claims, and the remarks made herein, the rejections under 35 U.S.C. §112, second paragraph, are overcome.

The previously recited limitation "said at least one component" has been rewritten to read "said at least one compartment". This element has a proper antecedent basis in the claim, and Applicant apologizes for the previous typographical error.

With regard to the Examiner's rejection relating to "allowing metabolite transport through the diffusion barrier and/or through at least one opening in the diffusion barrier", Applicant respectfully submits that the Examiner's objection is unclear and requests clarification. If the clarity problem arises from the fact that the metabolite transport may be through the diffusion barrier as such, or through holes or openings in the barrier, then the Examiner's attention is brought to the definition of diffusion barrier set forth at page 12, line 28, wherein it is explained that a diffusion barrier is understood to include both impermeable material which restricts the diffusive flow of metabolites to the metabolizing particle, wherein the transport of metabolites is through a hole in the material, as well as to material which is permeable to the metabolites and in which the metabolite passes therethrough by molecular diffusion. Accordingly, Applicant submits that it is not necessary for a diffusion barrier to include a hole. It is sufficient that the barrier be capable of allowing passage of the metabolite. If the Examiner deems it valuable to discuss this point further, Applicant would be very glad to speak directly with the Examiner and/or provide declaration testimony.

In view of the foregoing, Applicant respectfully submits that the rejections under 35 U.S.C. §112 are overcome.

Discussion of Metabolite Concentration Gradient

Before discussing the prior art in detail Applicant suggests that it may be valuable to discuss the principle of diffusion and a metabolite gradient.

Metabolite transport, such as oxygen transport, through an aquatic medium to a metabolizing particle can occur by two processes:

1) molecular diffusion of oxygen

2) mass transport, i.e. liquid movement of media carrying oxygen (convective transport).

The latter process - liquid movement - is usually orders of magnitude larger than the molecular diffusion in waterbodies that may be stirred by movement, surface wind, temperature differences, salinity differences etc.

Diffusion of a metabolite is the principle of movement of the metabolite without mass transport of the medium in which the metabolite is located.

As described at page 12, lines 24-26, diffusion is: "..The process whereby particles of liquids, gases, or solids intermingle as the result of random molecular motions caused by thermal agitation, resulting in a net transport of dissolved substances from a region of higher to one of lower concentration."

When the metabolite is moved as a result of diffusion then a metabolite concentration gradient is established. A diffusion gradient or concentration gradient is a gradient in concentration of a substance as a function of distance through a medium; and the movement of the substance down its concentration gradient is thus called diffusion.

The principle of diffusion is also given by Fick's law (see also page 45 of the description), basically giving the rate of diffusion of a substance through a given medium:

$$\text{Fick's law:} \quad dQ/dt = D \times A \times dC/dx,$$

where dQ/dt is the rate of diffusion, D is the diffusion coefficient, which is a characteristic of the medium and varies exponentially with temperature, A is the cross sectional area, and dC/dx is the concentration gradient over the diffusion distance.

Examples of metabolite diffusion gradients are shown in the present application, for example in figure 1 showing an O_2 concentration gradient established, and figure 5B showing a two-dimensional gradient established.

The requirements for allowing a metabolite concentration gradient to be established in a medium are that no mass transport of the medium takes place, and thereby that the metabolite transport is through diffusion only. When no mass transport of the medium takes place, then the medium is at standstill or stagnant. Stagnant is defined in the application page 16, lines 1-2, wherein a stagnant liquid is defined as: "A liquid without any flow, turbulence or movement..."

Oxygen transport through stagnant media occurs by molecular diffusion if a concentration gradient is established in the medium. Oxygen transport through a stagnant media can be accurately estimated based on the molecular diffusion coefficient of oxygen and the steady state concentration gradient in the media.

Thus, any attempts to move the medium, being through agitation, mixing or merely by convection destroy the standstill and thereby destroy the prerequisite for a concentration gradient to exist.

Apart from actively moving the media, for example by agitation as discussed in Miltenburger et al., mass transport and other influences on metabolite concentration may be caused by the presence of a liquid/air interface due to:

1) evaporative cooling, which may increase the density of the surface liquid and cause it to sink below thus effectively mixing the liquid phase
2) evaporative increase in the concentration of solutes near the surface which may also increase the density of the surface liquid and cause mixing
3) air currents over the liquid surface may induce liquid mixing.

Furthermore, a larger volume of liquid will always be more prone to mechanical mixing by movement and vibrations than a smaller liquid volume with a higher surface to volume ratio.

It has been found that a sufficiently small medium compartment, in particular sufficiently small with respect to surface area of the medium, will allow elimination of the causes for mass movement and any metabolite change not due to diffusion, whereby a sufficiently small medium compartment will allow a metabolite concentration gradient to be established.

Present claim 1 has been amended by including an upper limit for the transverse dimensions of a compartment, thereby providing an upper limit for the surface area of the medium. In particular when the transverse dimension of the compartment is less then 1.5 mm then a metabolite concentration gradient will be established when no active movements of the medium is made.

Claim rejections – 35 U.S.C. §102

Miltenburger et al. (US 4,649,114)

Miltenburger et al. describes active movement of the medium

Miltenburger et al. describes a fermentation apparatus, wherein a high concentration of metabolites in the fermentation vessel is desirable. At the same time, air bubbles should be avoided. Metabolites are hence provided to the medium through a gas permeable membrane. In order to improve the growth conditions in the fermentation vessel, the metabolites should preferably be distributed homogeneously throughout the medium in the vessel. The device according to Miltenburger is therefore equipped with propelling means to ensure an efficient mixing of the metabolite in the medium and an efficient transport of the metabolites to the cells. In col. 2, line 64 to col. 3, line 1 is specifically described that each volume on the microliter scale will contain the same amount of oxygen. The transport of the metabolites in the medium is not governed by diffusion but by flow induced mixing and Miltenburger et al. does neither explicitly

nor implicitly disclose a device wherein a metabolite diffusion or concentration gradient is established throughout the medium in the compartment.

Thus, Miltenburger et al. provides a compartment ensuring the opposite of the present invention, namely that due to mixing the same concentration of oxygen is ensured anywhere in the compartment, whereas the present invention ensures that the concentration differs according to the metabolite concentration or diffusion gradient.

Miltenburger et al. does not provide a chamber having a transverse dimension of 1.5 mm

Nowhere in Miltenburger et al. is a transverse dimension of the chamber provided, however it is evident from studying the figures of Miltenburger et al. that the dimensions of the compartment of the fermentation vessel are much more than 1.5 mm, in particular when seeing the relative dimensions in relation to the propeller and motor of the fermentation vessel.

Accordingly, the fermentation vessel of Miltenburger et al. has a different structure than the device according to the present invention, at least with respect to two features, in that Miltenburger et al. includes

- means for actively moving the medium (propeller)
- dimensions of the compartment much higher than 1.5 mm in any dimension.

Therefore, the independent claims and all dependent claims of the present invention are novel in view of Miltenburger et al.

Wodnicka et al. (J. Biomolecular screening, 2000)

Wodnicka et al. does not perform measurement of a metabolic rate

Wodnicka et al. describes a fluorescence based method for monitoring the number and viability of a **large number** of cells positioned in microplate wells (>1500 cells/well; p. 142, left col., line 14 from below). A fluorescent compound that is sensitive to the oxygen concentration

is embedded in a silica rubber (p. 142, right col., lines 9-1 from below) that is attached to the bottom of the microplate wells. Since the oxygen level is correlated to the number and viability of the cells, the fluorescence signal detected from the well provides information about the number of cells in that well and their viability.

Although Wodnicka et al. describes measurement of oxygen concentration this measurement is in order to obtain an estimate for the number of cells.

When measuring a metabolic rate the determination of the oxygen consumption requires very precise information about oxygen concentration and oxygen concentration gradient, whereas estimation of the number of cells in a compartment based on the oxygen consumption requires less precise information.

The wells are bigger than the compartment of the present invention

Wodnicka et al. describes three different multiwell plates, wherein the dimensions of the wells are as follows:

96-well U-bottom Falcon™

Diameter at bottom of each well 6.5 mm
Area= 33,2 mm2

24-well Falcon tissue culture plates

Diameter at bottom of each well 14 mm
Area= 153 mm2

384-well (Na/Ge Nunc International)

Diameter at bottom of each well 3.5 mm
Area= 9,6 mm2

Accordingly, none of the multiwell plates used in Wodnicka et al. has a transverse dimension less than 1.5 mm.

There is hence no disclosure in Wodnicka et al. of a device having a transverse dimension less than 1.5 mm, and wherein a metabolite diffusion gradient is established throughout the medium. The independent claims and all dependent claims of the present invention are hence novel in view of Wodnicka et al.

Conclusion relating to Claim rejections – 35 U.S.C. §102

The Examiner has stated that the establishment metabolite diffusion gradient is a functional limitation which does not materially change the structure of the claimed invention, however as discussed above this is not correct, since the establishment of a metabolite diffusion gradient most certainly depends on structural features, and therefore the functional limitation implies structural features.

Furthermore, also as discussed above Miltenburger et al. differs in structure from the present device both with respect to dimensions and with respect to the mixing means, and Wodnicka et al. differs in structure from the present device by having larger dimensions than the present device.

Accordingly, none of the references discloses devices having a dimension allowing a metabolite diffusion gradient to be established.

The Rejections under 35 U.S.C. §103

Pending claims were rejected under 35 U.S.C. §103 as being obvious in view of the Miltenburger '114 patent taken either singly or in combination with the Schwarz et al. publication. In view of the present amendments and remarks, the subject invention is now clearly distinguished over the teaching of Miltenburger and in view of the general inapplicability of the Miltenburger reference, the rejections under 35 U.S.C. §103 are likewise overcome. In summary, Miltenburger nowhere shows or suggests any apparatus in accord with the presently

claimed invention, nor does it show or suggest any modifications to the apparatus thereof which would cause it to approximate the present invention. In view thereof, Applicant respectfully submits that the rejections under 35 U.S.C. §103 are likewise overcome.

Conclusion

In view of the amendment and remarks presented herein Applicant respectfully submits that all claims are in condition for allowance. Should the Examiner feel that the application is not in condition for allowance Applicant respectfully requests that the Examiner accord the Applicant the opportunity to conduct an interview and/or submit expert testimony in the form of a declaration better explaining the distinctions of the present invention over the prior art.

Any questions, comments or suggestions the Examiner may have should be directed to the undersigned attorney.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 07-1180.

Dated:

Respectfully submitted,

By_____
Ronald W. Citkowski
Registration No.: 31,005
GIFFORD, KRASS, SPRINKLE, ANDERSON & CITKOWSKI, P.C.
2701 Troy Center Drive, Suite 330
Post Office Box 7021
Troy, Michigan 48007-7021
(248) 647-6000
(248) 647-5210 (Fax)
Attorney for Applicant